

PURIFICATION OF RECOMBINANT eIF-5A PRECURSOR PROTEIN AND
PREPARATION OF ITS DEOXYHYPUSINE INTERMEDIATE

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Eukaryotic initiation factor-5A (eIF-5A) is the only known protein in eukaryotic cells that contains the unusual amino acid hypusine (N^ε-(4-amino-2(R)-hydroxybutyl)lysine). The synthesis of hypusine, and therefore of mature eIF-5A, is important for its biological activity. Hypusine synthesis in the eIF5A is a unique two-step posttranslational modification catalyzed by deoxyhypusine synthase (DHS) and deoxyhypusine hydroxylase (DHH). Inhibitors of either the DHS or DHH have been shown to exert antiproliferative and antiretroviral effects. Therefore, these two enzymes are regarded as potential targets for anti-proliferative therapy. Since DHH has not yet been purified to homogeneity, the long-term objective of the research is to purify and characterize the enzyme. A detailed knowledge of structure-function relationship of this enzyme will help in designing specific inhibitors.

eIF-5A precursor protein is essential in developing assays and purification strategies for DHH. This project is aimed at purification of a recombinant eIF-5A precursor protein and preparation of deoxyhypusine form of eIF-5A. The recombinant glutathione-S-transferase (GST)-eIF5A fusion protein has been purified to homogeneity by an affinity chromatography. The GST carrier has been removed by thrombin digestion followed by affinity chromatography. The success of purification has been demonstrated by SDS-polyacrylamide gel electrophoresis. The deoxyhypusine form of eIF5A intermediate has been prepared from the eIF5A precursor by deoxyhypusine synthase catalysis. This intermediate has been linked to Affi-gel 102 which will be used to purify bovine deoxyhypusine hydroxylase.

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